Parenteral nutrition selectively decreases protein synthesis in the small intestine

MARY A. DUDLEY,1 LINDA J. WYKES,2 ALDEN W. DUDLEY, J. R.,3 DOUGLAS G. BURRN,1 BUFORD L. NICHOLS,1 JUDY ROSENBERGER,1 FAROOK JAHOOR,1 WILLIAM C. HEIRD,1 AND PETER J. REEDS1

1United States Department of Agriculture/ Agricultural Research Service, Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children’s Hospital, Houston, Texas 77030; 2School of Dietetics and Human Nutrition, McGill University, Montreal, Canada H9X 3V9; and 3Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center and New Jersey School of Dentistry and Medicine, Newark, New Jersey 07103

Dudley, Mary A., Linda J. Wykes, Alden W. Dudley, J. R., Douglas G. Burrin, Buford L. Nichols, Judy Rosenberger, Farook Jahoor, William C. Heird, and Peter J. Reeds. Parenteral nutrition selectively decreases protein synthesis in the small intestine. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G131–G137, 1998.—We investigated the effects of an elemental diet fed parenterally or enterally on total mucosal protein and lactase phlorizin hydrolase (LPH) synthesis. Catheters were placed in the stomach, jugular vein, and carotid artery of 12 3-day-old pigs. Half of the animals were given an elemental regimen enterally and the other half parenterally. Six days later, animals were infused intravenously with [3H]leucine for 6 h and killed, and the midjejunum of each animal was collected for analysis. The weight of the midjejunum was 8 ± 1.5 and 17 ± 1.6 g in parenterally fed and enterally fed piglets, respectively. LPH activities (µmol·min⁻¹·g protein⁻¹) were significantly higher in parenterally vs. enterally fed piglets. Total small intestinal LPH activities were lower in parenterally vs. enterally fed animals. The abundance of LPH mRNA relative to elongation factor-1α mRNA was not different between groups. The fractional synthesis rate of total mucosal protein and LPH was significantly lower in parenterally fed animals (67 ± 7 and 66 ± 7%/day, respectively) than in enterally fed animals (96 ± 7 and 90 ± 6%/day, respectively). The absolute synthesis rate (the amount of protein synthesized per gram of mucosa) of total mucosal protein was significantly lower in parenterally fed than in enterally fed piglets. However, the absolute synthesis rate of LPH was unaffected by the route of nutrient administration. These results suggest that the small intestine partially compensates for the effects of parenteral feeding by maintaining the absolute synthesis rate of LPH at the same levels as in enterally fed animals.

brush border; enteral; lactase phlorizin hydrolase

TOTAL PARENTERAL NUTRITION, introduced to clinical medicine about 30 years ago, is usually considered a major advance in the nutritional support of a wide range of patients who cannot tolerate enteral feeding. However, this form of nutritional support may have a profoundly negative effect on small intestinal mass and function, leading to an apparent decrease in digestive capacity (7, 17, 20, 24). The reduced digestive capacity appears to result in part from the reduced abundance of the brush-border (BB) hydrolases, which are responsible for the terminal stages of digestion (17, 24). In parenterally nourished rats, for example, total lactase phlorizin hydrolase (LPH) enzyme activity is 44% lower than that of enterally fed animals (20). Likewise, in humans, LPH enzyme activity is decreased fourfold by parenteral feeding (17).

LPH is an essential small intestinal digestive enzyme of newborn mammals and is responsible for the hydrolysis of lactose (the predominant sugar of mammalian milk) to glucose and galactose, which are in turn absorbed (13). Thus the synthesis and abundance of LPH are vitally important to the well-being of newborn animals, and a decrease in enzyme activity is of major physiological importance.

BB LPH is a glycoprotein synthesized only in the villus enterocyte. Enzyme synthesis is a complex process controlled by a series of transcriptional and post-transcriptional events that culminate in insertion of the mature protein into the BB membrane (8–10, 12, 13). Gene transcription starts in the cells at the base of the villus (12). The first detectable precursor form of the enzyme is translated and cotranslationally glycosylated in the endoplasmic reticulum to form a highmannose LPH precursor (proLPHH; Ref. 8). In the Golgi apparatus, proLPHH is converted to the complex glycosylated precursor (proLPHC), which in turn is translocated to the BB membrane (8). Either during translocation or immediately after insertion of the enzyme into the BB membrane, proLPHC is proteolytically cleaved to form the mature BB protein (8). Thus, as a result of its complex biosynthesis, LPH expression in the BB membrane can potentially be controlled by factors that regulate mRNA abundance and/or the rate of multiple steps of posttranscriptional synthesis.

Previous comparisons of parenteral vs. enteral feeding on intestinal morphology and function have generally compared parenteral administration of an elemental diet with enteral administration of a complex diet, making it impossible to distinguish between the effects of the route of dietary administration vs. the nature of the diet (15, 16). Furthermore, these studies have not examined the kinetics of synthesis of an essential digestive protein such as BB LPH. The present study was designed to examine in neonatal pigs the effect of parenteral vs. enteral administration of an elemental diet on mucosal protein synthesis in general, BB LPH activity, and the steps of BB LPH synthesis. We hypothesized that the rate of small intestinal protein synthesis is determined in part by the route of nutrient administration and that a decrease in BB LPH synthe-
sis might result from a decrease in the posttranslational processing rate of precursor LPH polypeptides.

**MATERIALS AND METHODS**

**Materials**

All amino acids for the elemental diets were obtained from Ajinomoto (Raleigh, NC). Intralipid (20%) and dextrose were obtained from Baxter-Travenol (Deerfield, IL), and Polycose was obtained from Ross Laboratories (Columbus, OH). Solutions of calcium gluconate, potassium phosphates, potassium was obtained from Baxter-Travenol (Deerfield, IL), and Polycose was obtained from Ross Laboratories (Columbus, OH). Solutions of calcium gluconate, potassium phosphates, potassium was obtained from Baxter-Travenol (Deerfield, IL). The MVI pediatric vitamin and multitrace pediatric mineral solutions were obtained from Rorer Group (Fort Washington, PA). L-[3,3,3-2H3]leucine ([2H3]leucine) was purchased from Cambridge Isotope Laboratory (Andover, MA). Dulbecco's phosphate-buffered saline was obtained from GIBCO (Grand Island, NY), and leupeptin, aprotinin, and antipain were obtained from Sigma (St. Louis, MO). Ulpure hydrochloric acid (12 M) was purchased from J. T. Baker Chemical (Phillipsburg, NJ). All other chemicals were of the highest analytical grade available. All aqueous solutions were prepared with deionized water (Millipore, Bedford, MA).

**Experimental Design**

The protocol for these studies was approved by the Animal Care and Use Committee of Baylor College of Medicine and was carried out in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS publication no. (NIH) 85–23, revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

Twelve 3-day-old piglets (2 animals from each of 6 litters) were obtained from the commercial swine herd at Texas A&M University, College Station, TX, and were housed individually in rooms with an ambient temperature of 25°C. The animals were anesthetized with 5% isoflurane (Aerrane, Anaquest, Washington, PA). L-[3,3,3-2H3]leucine ([2H3]leucine) was purchased from Cambridge Isotope Laboratory (Andover, MA). All other chemicals were of the highest analytical grade available. All aqueous solutions were prepared with deionized water (Millipore, Bedford, MA).

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Piglets were fitted with a jacket to house the catheters and to facilitate administration of the elemental diets through a tether/swivel apparatus (31–33). Piglets were allowed to recover from surgery for 3 h and were divided by weight into two dietary treatment groups. That is to say, the heaviest piglet was assigned to the first treatment group, the next two heaviest (animals 2 and 3) were assigned to the second treatment group, the next two heaviest (animals 4 and 5) were assigned to the first treatment group, the next two (animals 6 and 7) to the second treatment group, and so on. The final (lightest) animal was assigned to the first treatment group. One treatment group was fed the elemental diet via the jugular catheter (parenterally fed); the other was fed the elemental diet intragastrically (enterally fed).

As previously described, the elemental diets were designed to provide all the nutrients required by neonatal piglets for normal growth and tissue accretion (Table 1) (31–33). The parenteral and enteral infusates were identical, except that a short-chain glucose polymer (Polycose) was substituted for glucose as the carbohydrate source of the enteral infusate to reduce osmolality. The desired total energy intake of 220 kcal·kg⁻¹·day⁻¹ was achieved within 48 h of surgery. Twenty-six percent of energy was supplied by amino acids, 43% as glucose, and 31% as lipid. Macronutrient intakes were as follows (in g·kg⁻¹·day⁻¹): amino acids 13.5, glucose 25.4, and lipid 6.8.

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At the end of the infusion, the pigs were killed by intravenous injection of 0.33 ml/kg of Beuthanasia-D (Schering-Plough Animal Health, Kenilworth, NJ). The entire small intestine from the peritoneal reflection (analogous to the ligament of Treitz) to the ileocecal junction was excised immediately and placed in ice-cold saline. The small intestine was cut in half, and the proximal half was defined as the jejunum. The jejunum was then divided into thirds (proximal, mid-, and distal jejunum). Samples for mRNA analysis were taken from the middle portion of the midjejunum segment and immediately frozen in liquid nitrogen. Additional samples for histology were taken from the same location and placed in phosphate-buffered Formalin. The remainder of the midjejunal segment was then flushed with cold saline (9 g/l) and weighed. The mucosa was scraped, homogenized in phosphate buffer containing protease inhibitors (9), and frozen at –70°C until analyses were performed.

**Analyses**

Histological analysis. The jejunal samples were processed and stained with hematoxylin and eosin as previously described (13, 30). For each animal, 100 villi were measured, and the crypt depth was determined at 500 locations.

Measurement of LPH enzyme activity. LPH enzyme activity (µmol glucose·min⁻¹·g protein⁻¹) of mucosal homogenates was measured and converted to micromoles glucose per minute per gram mucosa as previously described (9, 12, 13). The latter value was then used to calculate the absolute synthesis rate (synthesis rate in 1 g of mucosa) of each LPH polypeptide (see below).

Measurement of steady-state mRNA abundance. RNA was isolated by the guanidine isothiocyanate-cesium chloride method and fractionated and blotted as previously described (13, 29). Radioactive probes for LPH mRNA and elongation

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**Table 1. Concentration of each nutrient in the elemental diet**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Parenterally Fed, µM</th>
<th>Enteraly Fed, µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid, g</td>
<td>53.3</td>
<td>53.3</td>
</tr>
<tr>
<td>Taurine, mg</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>70% Dextrose monohydrate, ml</td>
<td>143</td>
<td>91</td>
</tr>
<tr>
<td>Polycose, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium, mmol</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Potassium, mmol</td>
<td>15.1</td>
<td>15.1</td>
</tr>
<tr>
<td>Magnesium, mmol</td>
<td>37.2</td>
<td>37.2</td>
</tr>
<tr>
<td>Chloride, mmol</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Phosphate, mmol</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Zinc, µmol</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Manganese, µmol</td>
<td>308</td>
<td>308</td>
</tr>
<tr>
<td>Iron dextran, mg</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

*Vitamins and trace minerals were supplied as previously described (31–33).*
factor-1α (EF-1α, a ribosomal binding protein previously shown to be unaffected by diet or the animal’s stage of development) mRNA were prepared by random-primed oligo-labeling of linearized plasmids (6, 13, 29). Blots were washed, and hybridization was detected as previously described (6, 13, 29). After autoradiography to visualize the positions of the bands, the relative abundance of LPH mRNA was quantified by cutting the bands representing LPH or EF-1α mRNA from the blot and measuring their radioactivity in a liquid scintillation counter. The abundance [counts per minute (cpm)] of LPH mRNA was then calculated relative to the abundance (cpm) of EF-1α mRNA.

Measurement of posttranslational synthesis rates. The immunoprecipitation and purification of LPH polypeptides using the hybridoma PBB3/7/3/2 has been described (8). The preparation of plasma and mucosal free amino acid pools and LPH polypeptides for gas chromatography-mass spectrometry (GCMS) analysis has also been described (10). Briefly, amino acids from the tissue free amino acid pools and the protein hydrolysates were converted to the n-propyl ester heptafluorobutylamide derivatives. GCMS was performed using methanol/negative chemical ionization, with helium as the carrier gas on a Hewlett Packard 5988A instrument linked to an HP 5890 hydrogen gas chromatograph. One-microliter samples were injected using a 30:1 split onto a Silica-based DB5 capillary column (30 m x 0.2 mm, 1-μm film thickness; J & W Scientific, Folsom, CA). Chromatography was effected with a linear temperature gradient (80–250°C at 10°C/min). Isotopic abundances of ions at mass-to-charge ratios of 349, 350, and 352 were converted to tracer-to-tracee ratios using the matrix method (18).

Calculations

The fractional synthesis rate of mature BB LPH synthesis. In previous studies with anesthetized animals, we were able to obtain multiple tissue samples during an infusion and could therefore subject the kinetic data to compartmental analysis (8, 9). The intent of the present study was to measure LPH synthesis in unanesthetized piglets being fed parenterally or enterally; thus, only a terminal tissue sample could be obtained. Therefore, the fractional synthesis rates (FSR) of both total mucosal protein and BB LPH synthesis were calculated using the isotopic enrichment of proLPHh as the denominator in the simplified equation

\[
FSR (\%/day) = \frac{S_t}{S_i} \times \frac{24}{t} \times 100
\]

in which FSR is the fractional synthesis rate of total mucosal protein or BB LPH synthesized, \(S_i\) is the tracer-to-tracee ratio of \([2H_3]\)leucine in total mucosal protein or BB LPH after 6 h of infusion, \(S_t\) is the tracer-to-tracee ratio of \([2H_3]\)leucine in proLPHh, (the first detectable LPH precursor polypeptide synthesized) after 6 h of infusion, and t is labeling time, expressed in hours. We have previously used this same method to estimate FSR in conscious, unrestrained 2-wk-old pigs (10, 13).

The use of the equation involves two assumptions. First, as we have shown in rats and pigs (8–10), isotopic equilibrium is achieved rapidly in both the mucosal free amino acid pool and proLPHh. Second, once isotopic equilibrium is achieved in proLPHh, label incorporation into the BB protein is linear. Both assumptions have been shown to be valid (8–10). However, we have also shown a delay of ~1 h as label moves from proLPHh to proLPHc and finally to the mature BB protein. Thus, for both treatment groups, estimating the FSR of BB LPH from 0 h results in a slight underestimate of the true rate of label incorporation into the BB protein and hence the true FSR.

Abundance of mucosal protein. The abundance (g protein/g mucosa) of mucosal protein was determined as previously described (8).

Abundance of mature BB LPH. The abundance of BB LPH [arbitrary units (AU)] was estimated as previously described (8, 13). Briefly, for each animal, LPH enzyme activity (µM glucose·min⁻¹·g mucosa⁻¹) was divided by the relative abundance of BB LPH protein [i.e., the proportional contribution of the Coomassie blue-stained 160-kDa band (mature BB LPH) relative to the total amount of all Coomassie blue-stained LPH polypeptides observed by gel scanning (Table 2)] to yield, in AU, the total quantity of BB LPH protein in 1 g of mucosa. These values were then used to calculate absolute and total synthesis rates (see below). The total abundance of BB LPH in the midjejunal segments was calculated as the abundance in 1 g of mucosa multiplied by the mass of the mucosa.

The calculation of the abundance of BB LPH is based on the assumption that LPH enzyme activity is attributable only to the mature BB form of the enzyme. Naim et al. (21) have reported that the precursor forms of the enzyme appear to be enzymatically active in COS-1 cells transfected with the human cDNA for LPH. However, in COS-1 cells, the mature BB form of the enzyme seen in vivo is not synthesized; rather, a precursor form of LPH appears to be expressed at the cell surface. Because it has not been conclusively proven that the precursor polypeptides in the pig are enzymatically active, it seems reasonable to omit these proteins in calculations of total abundance. More importantly, the small difference in the relative amount of BB LPH between the two treatment groups (~1.25%) is not sufficient to significantly alter the calculations of BB LPH absolute synthesis rates (synthesis rate per gram of mucosa; see below).

Absolute synthesis rates. The absolute synthesis rate (synthesis per gram of mucosa, in AU) of total mucosal protein and mature BB LPH was calculated as the product of the abundance of the protein per gram of mucosa (above) and FSR (expressed per day as previously described; note, not %/day) (8, 13).

Total synthesis rates. The total synthesis rates (synthesis per segment of intestine, in AU) of mucosal protein and BB LPH in the midjejenum were calculated as the product of their absolute synthesis rate and the mass of the segment of small intestine (8, 13).

Statistics. Data are means ± SE. Differences between means were determined using an unpaired Student's t-test. P values <0.05 were considered statistically significant.

Table 2. Brush-border LPH enzyme activities, mRNA ratios, and polypeptide relative abundance in midjejenum in parenterally fed and enterally fed piglets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parenterally Fed</th>
<th>Enteraly Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPH enzyme activity (AU)</td>
<td>281 ± 33</td>
<td>182 ± 12</td>
</tr>
<tr>
<td>LPH total enzyme activity (AU)</td>
<td>94 ± 17</td>
<td>185 ± 29</td>
</tr>
<tr>
<td>LPH mRNA/EF-1α mRNA</td>
<td>131 ± 9</td>
<td>148 ± 14</td>
</tr>
<tr>
<td>LPH polysaccharide relative abundance</td>
<td>4.33 ± 0.34</td>
<td>2.93 ± 0.48</td>
</tr>
<tr>
<td>ProLPHh (%)</td>
<td>0.59 ± 0.08</td>
<td>0.80 ± 0.11</td>
</tr>
<tr>
<td>BB LPH (%)</td>
<td>95.11 ± 0.35</td>
<td>96.27 ± 0.35</td>
</tr>
</tbody>
</table>

Data are means ± SE. LPH, lactose phosphoril hydrolase; EF-1α, elongation factor-1α; proLPHh, high-mannose LPH precursor; proLPHc, complex glycosylated precursor; BB, brush border. *P < 0.05.
RESULTS

There were no differences in body weight between the two treatment groups, either at the beginning of the study or at its termination (mean initial weight and final weight for parenterally fed animals were 1.7 ± 0.2 and 2.4 ± 0.2 kg, respectively; initial and final weights for enterally fed animals were 1.7 ± 0.2 and 2.5 ± 0.2 kg, respectively). Histologically, the villi were significantly (P < 0.05) shorter in parenterally fed animals (240 ± 31 µm) than in those fed enterally (356 ± 16 µm). No differences in crypt depth were observed (128 ± 4 and 135 ± 8 µm for parenterally fed and enterally fed pigs, respectively). However, the jejunal enterocytes appeared to be morphologically normal in both dietary treatment groups. The cells were columnar, with nuclei located close to the basolateral membrane.

The weight of the midjejunum was significantly (P < 0.05) lower in parenterally than in enterally fed animals (8 ± 1.5 and 17 ± 1.6 g, respectively). However, neither the weight of the scraped mucosa as a percentage of the weight of the intestinal sample nor the protein content of the mucosa differed. In parenterally fed animals, the weight of the mucosa was 5.5 ± 0.9 g, or 69 ± 1% of the weight of the jejunal segment. In enterally fed animals, the weight of the mucosa was 13.5 ± 1.7 g, or 77 ± 4% of the weight of the jejunal segment. The protein concentration of the mucosa of the parenterally fed animals was 90 ± 5 mg protein/g mucosa; that of the enterally fed group was 95 ± 5 mg protein/g mucosa. LPH enzyme activity (µmol·min⁻¹·g protein⁻¹) was, however, significantly higher (P < 0.05) in the parenterally fed group than in the enterally fed group, whereas total LPH activity (µmol·min⁻¹·jejunal segment⁻¹) was significantly lower (P < 0.05) in the parenterally fed group (Table 2).

The jejunal RNA concentration was 1.6 ± 0.40 and 1.4 ± 0.35 mg/g jejunum in parenterally and enterally fed animals, respectively. EF-1α mRNA levels did not differ between groups (data not shown). As shown in Table 2, LPH mRNA abundance relative to that of EF-1α also did not differ.

After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, LPH polypeptides immunisolated from the solubilized, scraped mucosa separated into four bands, which we have previously identified as two precursor forms of BB LPH (proLPHh, 200 kDa; proLPHc, 220 kDa) and two forms of BB LPH: the 160-kDa polypeptide and a dimer of BB LPH with an apparent molecular mass of ~240 kDa (Fig. 1) (8, 10, 13). In order of synthesis, the LPH polypeptides are proLPHh (the first detectable translation product), proLPHc (the complex glycosylated precursor), and, finally, the mature BB enzyme (8, 10, 13).

The relative abundance of proLPHh (i.e., the abundance of proLPHh relative to the total abundance of all four LPH polypeptides on a gel) was significantly (P < 0.05) higher in the parenterally fed than in the enterally fed animals (Table 2). The relative abundance of proLPHc, and BB LPH was not significantly different between groups (Table 2).

The tracer-to-tracee ratios of the plasma free leucine pools of both treatment groups reached steady state within 1 h and did not differ at steady state (Table 3). However, the tracer-to-tracee ratio of the mucosal free leucine pools of the parenterally fed animals after 6 h of infusion was significantly higher (P < 0.05) than that of the enterally fed animals (Table 3). Thus the tracer-to-tracee ratio of the mucosal free leucine pool was 69 ± 7% of the plasma ratio in parenterally fed animals and 30 ± 7% of the plasma ratio in the enterally fed animals.

The tracer-to-tracee ratios of mucosal protein-bound leucine did not differ between treatment groups after 6 h of infusion (Table 3). However, the tracer-to-tracee ratio of protein-bound leucine expressed as a percentage of the corresponding mucosal free leucine pool was lower in parenterally fed animals (17 ± 0.8%) than in enterally fed animals (46 ± 5%). The tracer-to-tracee ratio of leucine in the earliest detectable precursor polypeptide (proLPHh) tended (P = 0.066) to be higher in parenterally fed than in enterally fed animals (Table 3). The tracer-to-tracee ratio of proLPHh-bound leucine expressed as a percentage of the tracer-to-tracee ratio of plasma free leucine was significantly (P < 0.05) higher in the parenterally fed than in enterally fed animals (72 ± 5% and 45 ± 6.5%, respectively).

Table 3. Tracer-to-tracee ratio of leucine in plasma and mucosal free amino acid pools, mucosal protein and LPH polypeptides after 6-h infusion of [3H]leucine

<table>
<thead>
<tr>
<th></th>
<th>Parenterally Fed</th>
<th>Enterally Fed</th>
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<tbody>
<tr>
<td>Plasma free leucine pool</td>
<td>4.4 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Mucosal free leucine pool</td>
<td>3.0 ± 0.2</td>
<td>1.5 ± 0.4*</td>
</tr>
<tr>
<td>Mucosal protein-bound leucine</td>
<td>0.52 ± 0.05</td>
<td>0.50 ± 0.07</td>
</tr>
<tr>
<td>ProLPHh-bound leucine</td>
<td>3.1 ± 0.12</td>
<td>2 ± 0.41</td>
</tr>
<tr>
<td>ProLPHc-bound leucine</td>
<td>1.9 ± 0.2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>BB LPH-bound leucine</td>
<td>0.51 ± 0.07</td>
<td>0.49 ± 0.08</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05; † P = 0.066.
tracer-to-tracee ratios of proLPHc-bound leucine did not differ between groups (Table 3). The tracer-to-tracee ratio of proLPHc, expressed as a percentage of the tracer-to-tracee ratio of proLPHa, also did not differ between groups (61 ± 5% in parenterally fed animals and 73 ± 5% in enterally fed animals). As expected from previous studies (8, 10, 13), the tracer-to-tracee ratio of BB LPH-bound leucine did not reach steady state during the 6-h infusion (Table 3). As previously discussed, the tracer-to-tracee ratios in the 240-kDa dimer paralleled those of BB LPH (data not shown) (8, 10, 13).

The synthesis rates of total mucosal protein and BB LPH are shown in Fig. 2. The FSR of both total mucosal protein and BB LPH was significantly lower in parenterally fed (67 ± 7%/day, mucosal protein; 66 ± 1%/day, BB LPH) than in enterally fed animals (96 ± 7%/day, mucosal protein; 90 ± 1%/day, BB LPH).

The absolute synthesis rate (a measure of the amount of protein synthesized per gram of mucosal tissue) of total mucosal protein was significantly (P < 0.05) lower in parenterally fed (57 ± 4 AU) than in enterally fed animals (89 ± 8 AU). The total abundance of BB LPH in the midjejunum was significantly lower (P < 0.05) in parenterally fed than in enterally fed animals (97 ± 17 and 177 ± 31 AU, respectively). However, the absolute synthesis rate of BB LPH did not differ between the two treatment groups (on average, 12 ± 1.3 AU for both groups).

The total synthesis rate (a measure of the amount of protein synthesized in the midjejunal segment) of mucosal protein, as well as that of BB LPH, was significantly lower (P < 0.05) in parenterally fed piglets (317 ± 58 and 57 ± 6 AU for total mucosal protein and BB LPH, respectively) than in enterally fed piglets (1,190 ± 175 and 159 ± 25 AU for mucosal protein and BB LPH, respectively).

DISCUSSION

Parenteral nutrition has been associated with adverse effects on the small intestine in a wide variety of mammalian species (1, 3, 7, 14–16, 19, 20, 24–27). In adult laboratory animals without apparent intestinal disease, parenteral administration of an elemental diet seems to result in a pronounced reduction of intestinal mass, in decreased disaccharidase enzyme activities, and in altered intestinal morphology (3, 7, 14, 15, 24). In adult humans, the specific consequences of parenteral nutrition are extremely varied, perhaps because for most studies patients with intestinal diseases have been examined. In patients receiving parenteral nutrition for treatment of postoperative enterocutaneous fistulas, for example, intestinal morphological changes were observed (23). In contrast, in patients with inflammatory bowel disease, parenteral nutrition did not affect intestinal morphology but decreased BB hydrodase activities (17). Results from the only study that, to our knowledge, has examined the effect of parenteral nutrition in normal volunteers demonstrated intestinal morphological and functional changes paralleling those reported in laboratory animals (1). Unfortunately, BB disaccharidase activities were not determined.

The effect of parenteral nutrition on intestinal development and function in young mammals has received less attention than its effect on adults. Several studies have examined the effect of elemental regimes (i.e., glucose alone vs. glucose plus fat) in very young laboratory animals (26, 27), but none have examined the effect of parenteral vs. enteral alimentation. To our knowledge, the only study in children compares the effect of parenteral nutrition in individuals with short bowel syndrome or inflammatory bowel disease with individuals complaining of abdominal pain and/or chronic diarrhea who were given a complex oral diet (25). Thus, it is not clear whether the effects of parenteral nutrition in the young parallel the effects in adults. Furthermore, the mechanisms underlying these effects have not been examined.

The present study in neonatal pigs was undertaken to examine the effect of an elemental diet administered parenterally or enterally on small intestinal mucosal protein synthesis in general and on the steps of synthesis of a specific intestinal protein (BB LPH) that is of vital importance to young animals. Piglets were chosen for the study because they are our best laboratory model for human intestinal development and function (2, 28). Furthermore, during infancy in piglets there is a rapid rate of somatic and splanchnic growth. This early rapid growth allows for the evaluation of the nutritional effects on the gastrointestinal tract over a short period of time. Of equal importance, by undertaking the study in apparently healthy neonatal animals, the effects of an elemental diet could be examined without the confounding effects of small intestinal disease or surgical resection.

Under the conditions of the present study, and as reported by other investigators, the route of nutrient administration had no effect on piglet growth as determined by whole body weight gain (20). However, the weight of the jejunum was significantly lower in parenterally fed than in enterally fed animals (20). Villus length was likewise significantly lower in parenterally fed than in enterally fed piglets, but crypt depth was
unaffected by the route of nutrient administration (1, 15, 23, 24).

Neither the protein nor the RNA concentration differed significantly between treatment groups. As reported in rats, LPH enzyme activity (expressed as μmol·min⁻¹·g protein⁻¹) was significantly higher in parenterally fed than in enterally fed animals (5). However, because of the lesser total mucosal mass in the parenterally fed animals, LPH total activity was significantly lower in parenterally fed than in enterally fed piglets.

The most striking and important finding in the present study was that the absolute synthesis rate (synthesis rate per gram of mucosa) of total mucosal protein and that of BB LPH appeared to be individually and differentially affected by the route of nutrient administration. The FSRs for total mucosal protein and BB LPH were on average 40% lower in parenterally fed than in enterally fed animals. The absolute synthesis rate of total mucosal protein was significantly lower in parenterally fed than in enterally fed animals. In contrast, the absolute synthesis rate of BB LPH did not differ between treatment groups. Because of the reduced size of the small intestine in the parenterally fed animals, the total synthesis rates (synthesis rate/midjejunal segment) of both total mucosal protein and BB LPH were lower than in enterally fed animals. These observations imply that LPH synthesis and presumably lactose digestion are less susceptible to the route of nutrient administration than overall intestinal mass and protein synthesis. Furthermore, the data suggest that although the small intestine cannot compensate for the intestinal “atrophy” resulting from parenteral nutrition, it partially compensates for the resulting reduced total abundance of at least one physiologically vital enzyme, BB LPH, by maintaining its absolute synthesis rate.

Similar examples of compensatory mechanisms in the small intestine have been reported in the the hibernating ground squirrel. In these animals, pronounced small intestinal atrophy and a significant decrease in total BB absorption of 3-O-methylglucose occur during hibernation (4). However, 3-O-methylglucose absorption per gram is significantly higher in hibernating than in active animals. Thus, although the synthesis rate of this transport protein has not been determined and the mechanism is undefined, it appears that in the hibernating ground squirrel the villus enterocyte compensates for the loss of total nutrient absorption resulting from a decrease in intestinal mass by upregulating the rate at which nutrients are absorbed in a unit of tissue. Note, however, that the findings in the present study are not strictly parallel to intestinal changes occurring in the hibernating ground squirrel, for we did not measure lactose absorption but rather LPH synthesis rates.

Although from this study we cannot precisely define the mechanisms underlying the changes in protein synthesis rates observed in parenterally fed animals, it is interesting to note that the route of nutrient administration appears to preferentially affect posttranscrip-

The effects of parenteral nutrition on LPH synthesis in the present study are consistent with our earlier studies in pigs and rats. We have shown, for example, that the posttranslational processing of sucrase-isomaltase is significantly higher in fed than in fasted animals (11). Furthermore, we have demonstrated that the nature of dietary nutrients appears to modulate the rate of posttranslational processing (8). It is noteworthy, however, that we have also shown that in enterally fed, protein-malnourished piglets, in contrast to these well-nourished but parenterally fed animals, LPH synthesis appears to be regulated by both LPH mRNA abundance and by the FSR of the mature BB protein from proLPHh (13). In the earlier study of protein malnutrition in enterally fed pigs, LPH mRNA abundance relative to EF-1α mRNA abundance was significantly lower in protein-malnourished than in well-nourished piglets (13). Posttranslational processing was likewise affected.

In summary, the results of the present study demonstrate that in young pigs, the small intestine can partially adapt to the absence of luminal nutrients by selectively maintaining the absolute synthesis rate of intestinal proteins. Under the conditions of the present study, intestinal adaptation appears to favor the synthesis of a protein responsible for the terminal stages of digestion at the expense of overall mucosal protein synthesis.

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Address for reprint requests: M. A. Dudley, Dept. of Pharmacology and Physiology, New J ersey School of Dentistry and Medicine, 185 South Orange Ave., Newark, NJ 07103.

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